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ORIGINAL PAPER

Binding free energy calculation with QM/MM hybrid methods for Abl-Kinase inhibitor

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Abstract We report a Quantum mechanics/Molecular Mechanics–Poisson-Boltzmann/Surface Area (QM/MM-PB/SA) method to calculate the binding free energy of c-Abl human tyrosine kinase by combining the QM and MM principles where the ligand is treated quantum mechanically and the rest of the receptor by classical molecular mechanics. To study the role of entropy and the flexibility of the protein ligand complex in a solvated environment, molecular dynamics calculations are performed using a hybrid QM/MM approach. This work shows that the results of the QM/MM approach are strongly correlated with the binding affinity. The QM/MM interaction energy in our reported study confirms the importance of electronic and polarization contributions, which are often neglected in classical MM-PB/SA calculations. Moreover, a comparison of semi-empirical methods like DFTB-SCC, PM3, MNDO, MNDO-PDDG, and PDDG-PM3 is also performed. The results of the study show that the implementation of a DFTB-SCC semi-empirical Hamiltonian that is derived from DFT gives better results than other methods. We have performed such studies using the AMBER molecular dynamic package for the first time. The calculated binding free energy is also in agreement with the experimentally determined binding affinity for c-Abl tyrosine kinase complex with Imatinib.

Keywords Molecular dynamics · QM/MM-PB/SA · Binding free energy · Tyrosine kinase · Semi-empirical methods

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1 Introduction

Biological functions are described by interactions of biological macromolecules like proteins, DNA, and RNA. Computer simulation techniques play a substantial role in providing theoretical approaches for exploration of mechanisms of biological systems. It is difficult to perform theoretical investigation of such biological molecules based on electronic contributions. Most proteins consist of several thousand atoms, and solvent water molecules contribute to the reaction as well as to stabilization of their 3D structure, leading to huge model systems for calculations. Therefore, for the study of structural and theoretical features of biological systems, molecular dynamics (MD) simulations using molecular mechanics (MM) potentials have usually been performed. Although this method is widely used and plays a vital role in understanding structural functions, it also possesses some limitations. These limitations occur due to neglect of electronic contributions that play a substantial role in processes like formation and/or cleavage of covalent bonds and fluctuation of charge during molecular dynamics. The quantum mechanical approach gives an insight to overcome this problem but it is not practical to apply this technique on entire biomolecular systems. Thus, Quantum Mechanics/Molecular Mechanics (QM/MM) hybrid calculations have been used in recent years to increase computational accuracy [1–4]. In this approach, we apply MM force fields to the receptor/protein molecule while QM potentials are used for the ligand/core molecules. The total energy function used in this approach is

$$E = E^{\mathrm{QM}} + E^{\mathrm{MM}} + E^{\mathrm{QM/MM}}.$$

Here, E^{QM} and E^{MM} are the energies for the QM and the MM regions, which are calculated using the selected QM methods and the usual force field equations, respectively. The last term, $E^{\mathrm{QM/MM}}$, describes the interaction between the QM and MM parts and typically contains the terms for electrostatic, van der Waals, and bonding interactions across the region. Several new techniques were proposed as the predictor of binding affinity for protein–ligand complexes [5–7]. Linear Interaction Energy (LIE) is a commonly used alternative rapid free energy pathway that involves running two MD simulations with an empirical force field: one for the ligand in solution and the other for the ligand at the protein binding site. The principle and applications of this technique may be found elsewhere [8–10]. Nonetheless, the quality of the results is somewhat surprising, because LIE does not account explicitly for the change in entropy or the internal energy of the ligand. Therefore, in the present study we have used Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PB/SA) method for binding free energy calculations.

The use of MM-PB/SA methods gives binding affinity of protein–ligand complexes but ignores the energy contributions due to the $E^{\mathrm{QM/MM}}$ term. In the present study, a combined QM/MM description is incorporated into free energy calculations of protein/ligand binding using the AMBER package [11]. The aim of study is to combine a quantum mechanical treatment for the inhibitor and the classical treatment for the protein with PB/SA free energy calculation scheme [12] and evaluating its reliability. The QM/MM [13] MD simulation using DFTB-SCC and various semi-empirical methods, PM3, MNDO, MNDO-PDDG, and PDDG-PM3, is followed by binding free-energy evaluation, which involves a quantum mechanical energy component accounting for ligand strain and protein ligand electrostatic interaction including polarization effects. In the present work, a well-studied ATP competitive inhibitor Imatinib, which is very effective and approved as a drug for cancer, when complexed with c-Abl tyrosine kinase, is used as model system.

2 Materials and methods

System setup for c-Abl complex The crystal structure of the c-Abl human tyrosine kinase complex with Imatinib (pdb code 2HYY), resolution of 2 Å, was used for calculations [14]. The parameters for the ligand were prepared by an ab initio method using Gaussian 03 at the 6–31G* level of theory. Partial atomic charges of the drug Imatinib are calculated using restrained electrostatic potential procedure [15–17]. The residue Glu 220 and heavy atoms of other residues missing in the PDB structure are added, and hydrogen atoms were incorporated using the AMBER package. After system preparation, MD simulations, using a combined quantum mechanical QM/MM methods were initiated [11]. The details of MD simulation methods may be seen in our earlier publications [18, 19] and in the supplementary information. Six simulations are carried out for this system. The protein and water were modeled classically using the AMBER force field with parameter set ff03, while the ligand was treated with semi-empirical QM methods DFTB-SCC [20], PDDG-PM3 [21], PM3 [22], MNDO [23], and MNDO-PDDG [21] as interfaced in AMBER 10. The DFTB-SCC [20] was proven useful for simulation of biomolecular systems with accuracy equivalent to ab initio methods.

QM/MM-PB/SA formalism The free energy is computed separately for ligand (L), protein (P), and ligand/protein complex (C). For each conformation of the species, the free energy (G) is decomposed here empirically. This decomposition has proven useful in free energy analysis [24, 25] and is discussed in literature [26, 27]:

$$G = \text{Intramolecular energy } (E^{\text{int}}) + \text{Solvation Free Energy } (G^{\text{sol}}) - TS^{\text{ideal}}.$$

The decomposed free energy for ligand, protein, and complex is as follows.

Unbound ligand free energy As the ligand is treated quantum mechanically, its intramolecular energy will correspond to E^{QM}. Therefore, the free energy for the ligand is given by

$$G(L) = E^{\text{QM}}(L^{\text{u}}) + G^{\text{sol}}(L^{\text{u}}) - TS^{\text{ideal}}(L^{\text{u}})$$

where the superscript u indicates the unbound conformation of the ligand.

Unbound protein free energy The protein is treated by classical mechanics during the simulation for the unbound protein. Its free energy is composed of total intramolecular energy, E^{int} [van der Waals interaction (E^{vdw}) + Coulombic interaction (E^{coul}) + bonding interaction (E^{bond})], solvation free energy (E^{solv}) and entropic contributions ($-TS^{\text{ideal}}$).

$$G(P) = E^{\text{int}}(P^{\text{u}}) + G^{\text{solv}}(P^{\text{u}}) - TS^{\text{ideal}}(P^{\text{u}}).$$

Complex free energy The complex is treated by both MM and QM methods, and it contains the sum of energy terms corresponding to the protein and ligand in the bound state and of the term for protein ligand interactions (P/L). The free energy of complex is composed of following terms:

$$G(C) = E^{\text{int}}(P^{\text{b}}) + E^{\text{QM}}(L^{\text{b}}) + G^{\text{solv}}(C^{\text{b}}) + \alpha\ E^{\text{vdw}}(P/L) - TS^{\text{ideal}}.$$

Here, E^{int} is decomposed as the sum of E^{bond}, E^{coul}, and E^{vdw} and the superscript b denotes the bound form. In the bound state, $E^{\text{QM}}(L^{\text{b}})$ contains the electrostatic protein/ligand interaction; hence, $\alpha E^{\text{elec}}(P/L)$ is included in $E^{\text{QM}}(L^{\text{b}})$.

The binding free energy for the noncovalent association of two molecules may be written as

$$\Delta G_{\text{bind}} = \Delta G\,(L + P \rightarrow C) = G\,(C) - G\ (L) - G\,(P)\ \text{or}$$

$$\Delta G_{\text{bind}} = \Delta E^{\text{int}} + \Delta E^{\text{QM/MM}} + \Delta G^{\text{solv}} - T\Delta S^{\text{ideal}} + \alpha \Delta E^{\text{vdw}}\,(P/L)$$

Here,

$$\Delta E^{\text{int}} = E^{\text{int}}\left(P^{\text{b}}\right) - E^{\text{int}}\,(P^{\text{u}})\,;$$

$$\Delta E^{\text{QM/MM}} = E^{\text{QM}}\,(L^{\text{u}}) - E^{\text{QM}}\left(L^{\text{b}}\right),$$

$$\Delta G^{\text{solv}} = G^{\text{solv}}\left(C^{\text{b}}\right) - G^{\text{sol}}\,(L^{\text{u}}) - G^{\text{solv}}\,(P^{\text{u}})\,,\ \text{and}$$

$$-T\Delta S^{\text{ideal}} = -TS^{\text{ideal}} + TS^{\text{ideal}}\,(L^{\text{u}}) + TS^{\text{ideal}}\,(P^{\text{u}})\,.$$

The empirical scaling factor α is introduced to balance the van der Waals energy relative to the electrostatic energy. It is obtained from the experimental results and here it is only used as a fitting parameter. ΔE^{int} is the change in protein intramolecular energy which is calculated by MM-PB/SA method [12] of AMBER package, $\Delta E^{\text{QM/MM}}$ is the interaction energy between the receptor and ligand obtained using the QM/MM approach. It is calculated by the SANDER module of the AMBER package by manual subtraction of the DFTB-SCC (or MNDO-PDDG, PDDG-PM3, PM3, MNDO) energy of the bound state of the ligand from the unbound state. ΔG^{solv} [12] is composed of nonpolar contribution and polar contribution. Nonploar solvation energy accounts for the unfavorable cavity formation and favorable van der Waals interaction between solute atoms and the solvent [9]:

$$\mathrm{G}^{\text{solv,np}} = \gamma \mathrm{A} + \mathbf{b}$$

where A stands for solvent accessible surface area (SASA).

γ is an empirical constant of 7.2 cal/(mol/Å^2) and the empirical constant **b** has a value of zero. The nonpolar solvation energy term was calculated by Molsurf [28] implemented in the AMBER package. The polar solvation energy was calculated using the linear Poisson–Boltzmann (PB) equation [29] that relates the charge density, $\rho(r)$, to the electrostatic potential, $\phi(r)$, in a medium with non-uniform dielectric permittivity $\varepsilon(r)$ which was set as 1 and 80 for the solute and the solvent, respectively

$$\Delta\varepsilon\,(r)\ \ \Delta\phi\,(r) = -4\pi\ \ \rho\,(r) + \kappa^2\ \varepsilon\,(r)\ \ \Delta\phi\,(r)$$

where κ is the Debye–Huckel screening parameter to take into account the electrostatic screening effect. The required partial atomic charges of the ligand were calculated by ab initio electrostatic surface potential calculations with HF/6–31G* level of theory. During the implementation of the QM/MM term for protein ligand interaction with the classical electrostatic solvation term, some contributions to the binding energy are neglected.

$T\Delta S$ arises from the change in translational, rotational and vibrational degrees of freedom in the system upon protein-ligand binding [30]. The Nmode module of the AMBER package usually calculates this contribution and it estimates the entropic contribution.

3 Results

In this section, we present the structural features of the c-Abl kinase inhibitor complex and report the results of the QM/MM-PB/SA analysis.

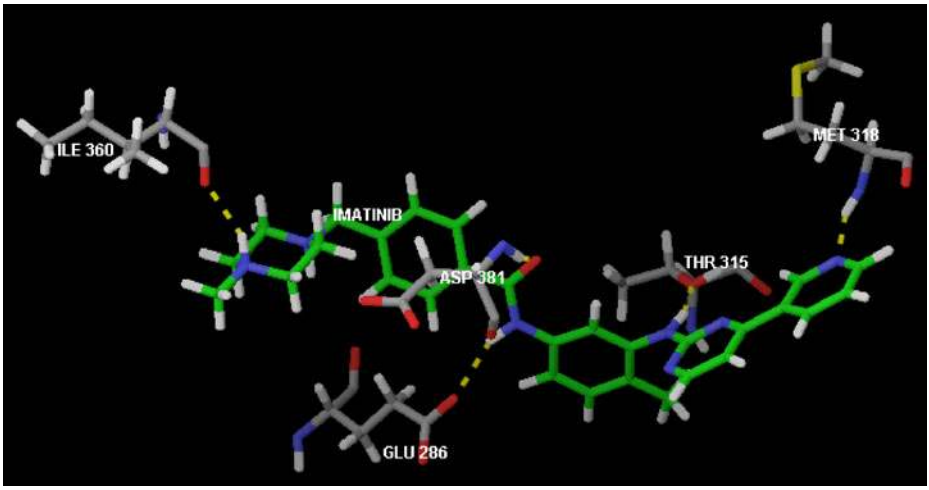

Fig. 1 Interactions of Imatinib with the tyrosine kinase receptor. Imatinib is shown in *green*, while the hydrogen bonds are shown with *yellow dotted lines*

3.1 Ligand active site interaction

c-Abl tyrosine receptor has a bilobal structure which is arranged into a C-terminal and an N-terminal domain. The drug, Imatinib, as expected, binds the kinase in a deep cleft formed by these two lobes [31–36]. Imatinib is surrounded by a large hydrophobic region of area approximately equal to 146.85 $Å^2$ and the hydrophilic region of area equal to 82.580 $Å^2$. The hydrophilic regions are surrounded by electronegative residues Glu 286, Thr 315, Ile316, Met 318, and Asp 381. On the other side, the hydrophobic pocket is surrounded by Lys 271, Met 290, Ile 316, Leu 379, and Asp 381. Imatinib forms five hydrogen bonds with the hydrophilic residues Glu 286, Thr 315, Met 318, Ile 360, and Asp 381. The interactions via hydrogen bonds with the receptor of Abl kinase are shown in Fig. 1. The hydrogen bond

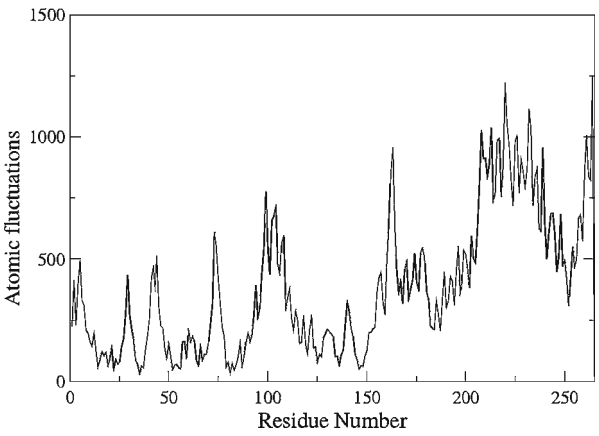

Fig. 2 Average residue fluctuations during the entire molecular dynamics simulation using DFTB QM method. The crystallographic structure is a tetramer; here, only the monomer was simulated; therefore, residue 1 in Fig. 2 corresponds to residue 235 of the crystal structure

distances of Tyr 253, Glu 286, Met 290, Thr 315, and Asp 381 during MD simulation are shown in Fig. 1S. The nitrogen N1 of the pyridine ring of STI-571 acts as a hydrogen bond donor to the amide nitrogen of Met 290. Similarly, OG1 atom of Thr 315 forms a hydrogen bond with the H30 atom of STI-571 and the OE2 atom of Glu 286 is a proton acceptor and forms a hydrogen bond with the H31 atom of STI. Besides these strong interactions via hydrogen bonds, many other contacts are also apparent with Tyr 253, Lys 271, Met 290, Phe 317, Ala 380, and Phe 382 as shown in Fig. 2S. The DFG loop (Asp 381, Phe 382, Gly 383) which plays a significant role in the activity of tyrosine kinase, shows a good contact with Imatinib through Asp 381. The molecular dynamics simulations show that residues 384–398 are very flexible (atomic fluctuations are shown in Fig. 2); these regions correspond to the activation loop whose importance in the activity of the kinase has already been discussed in previous works [37–39].

QM/MM-PB/SA results For the binding free energy calculations, we performed QM/MM-PB/SA simulations. Table 1 shows results obtained from binding free energy calculations using different QM potentials. The intramolecular (E^{int}) energy is composed mainly of van der Waals interactions for all QM potentials. It is clear that among all the interaction terms, E^{int} has the largest contribution. The relative nonpolar solvent free energy of the protein ligand complex formation is negative, which implies the favorable cavity formation and van der Waals interactions between Imatinib and c-Abl receptor. The binding free energy calculated by QM/MM-PB/SA methods contains E^{QM} energy, which has significant value for all QM potential methods. For the classical MM calculations, fitting the parameter α equals 0.10475 when using the AMBER force field [40]. We have used this value in our calculations and the binding energy for this value is represented by ΔG_1^{bind}. Usually, electronic contributions are not considered in MM calculations but for the QM calculations, due to electronic contributions, this parameter should be different. Therefore, we have considered a new value of fitting parameter α' as 0.1656 to achieve a calculated binding

Table 1 Binding free energy components of the c-Abl tyrosine kinase complex with Imatinib

Cont	DFTB	PDDG-PM3	PM3	MNDO	PDDG-MNDO
ΔE^{ELE}	−18.23 (5.49)	−26.50 (3.30)	−17.69 (3.67)	−14.06 (4.50)	−17.23
ΔE^{VDW}	−72.16 (1.53)	−74.87 (3.14)	−69.89 (3.69)	−72.98 (5.74)	−66.24
ΔE^{INT}	−90.39 (5.62)	−101.37 (4.22)	−87.58 (3.90)	−87.04 (8.39)	−83.48
ΔE^{SNP}	−8.71 (0.10)	−8.60 (0.18)	−8.64 (0.18)	−9.04 (0.12)	−8.83
ΔE^{SP}	51.36 (6.95)	61.36 (3.72)	55.42 (3.98)	53.40 (5.27)	60.10
ΔG^{SOL}	42.65 (6.92)	52.76 (3.66)	46.78 (3.95)	44.36 (5.25)	51.27
ΔG^{TOT}	−47.74 (7.98)	−48.61 (5.02)	−40.81 (3.39)	−42.68 (8.50)	−32.21
$\Delta E^{\text{QM/MM}}$	−14.73	−23.37	−14.23	−8.83	−15.62
$-T\Delta S^{\text{ideal}}$	64.05 (0.34)	62.62 (0.62)	61.35 (0.89)	63.79 (1.44)	63.84
$\alpha\Delta E^{\text{vdw}}(P/L)$	−7.55	−7.83	−7.31	−7.64	−6.99
ΔG_1^{bind}	−6.02	−17.19	−1.00	4.64	9.07
$\alpha'\Delta E^{\text{vdw}}(P/L)$	−11.94	−12.39	−11.53	−12.08	−10.96
ΔG_2^{bind}	−10.37	−21.75	−03.19	0.20	5.04

All values are measured in kcal/mol. Values in parenthesis are the standard error of the mean values

free energy close to the experimental value. Since DFTB is an ab initio method and it gives an approximate result, α' is calculated for DFTB method as follows:

$$\Delta G_{\text{exp}} \approx \Delta G_{\text{DFTB}} = \Delta G_{\text{cal}} + \alpha' \Delta E_{\text{vdw}} \, (P/L)$$

where ΔG_{cal} is sum of ΔG_{TOT}, $\Delta E^{\text{QM/MM}}$ and $-T\Delta S$. The binding free energy for this fitting parameter α' is represented by ΔG_2^{bind} in Table 1.

4 Discussion

The van der Waals energy, ΔE^{vdw}, was found to be the main energy term favoring the binding, due to its highest contribution, with the most favorable van der Waals energy of −74.87 kcal/mol for PDDG-PM3 calculation. The least favorable van der Waals contributions are found for MNDO-PDDG as shown in Table 1. The polar and nonpolar contributions to the solvation free energy also play a significant role in binding with ΔE^{SNP} ranging from −8.64 to −9.04 kcal/mol and ΔE^{SP} varying from 51.36 to 61.36 kcal/mol. Nonpolar solvation energy has a favorable role due to its negative value while the polar solvation energy has unfavorable binding role as it has a positive value. The nonpolar groups of ligand experience maximum exposure to the solvent. The total intramolecular interactions show higher contributions to the favorable binding of the ligand. The negative value of the intramolecular energy signifies that binding involves a decrease in the protein intramolecular energy; hence, it tends to be in a more stable conformation after binding. Entropic contributions due to changes in the translational, rotational, and vibrational degrees of freedom should be taken into account in binding free energy calculations as indicated by the broad range of $-T\Delta S^{\text{ideal}}$ from 61.35 to 65.53 kcal/mol during various simulations. All terms composing entropy of the complex formation have positive values, which show a tendency against binding due to protein flexibility during molecular dynamics. The comparison of results obtained by implementation of various QM potentials exhibits a similarity in the range of van der Waals energies and nonpolar solvation free energies that indicates that change of QM potential does not produce a significant effect on these terms. Since Coulombic interactions and polarization terms are a function of QM charge, variations in ΔE^{ELE} and ΔE^{SP} energy terms are well understood. ΔE^{QM} comprises the change in ligand intramolecular energy and the electrostatic interaction between protein and ligand. This large contribution of ΔE^{QM} to the binding free energy due to the electrostatic and polarization interactions of the ligand is surprising at a first glance. The highly negative value of $\Delta E^{\text{QM/MM}}$ favors the binding of the ligand. This negative value is largest for the PM3-PDDG potential and the smallest for the MNDO. The $\Delta E^{\text{QM/MM}}$ value for MNDO-PDDG potential, which is an improved AM1 potential, is −15.62 kcal/mol. According to Table 1, the binding free energy (ΔG_1^{bind}) calculated by DFTB-SCC QM/MM implementation is −6.02 kcal/mol which is nearest to the experimental value of −10.37 kcal/mol [41]. The MNDO QM potential shows the least negative QM/MM energy and a positive binding free energy, which indicates a clear dependency of binding free energy on the QM interaction energy. The modified fitting parameter α' does not change the order of accuracy but enhances the binding free energy (ΔG_2^{bind}) of each implementation method. Therefore, the inaccurate experimental determination of the fitting parameter may also cause an error in theoretical calculations of binding free energy.

5 Conclusions

Binding free energy calculations using classical MM-PB/SA methods are frequently used to calculate the binding free energy of protein-ligand complexes but the results of the calculations are less reliable due to the neglect of the electronic contributions. In the present study, contributions of electronic interactions and polarization effects due to the QM treatment are evident by the large value of ΔE^{QM}. Implementation of QM/MM techniques for protein-ligand complexes is a fertile field for computational research of biomolecular systems, but the implementation of this technique for the exact calculation of binding affinity is really a hard task. To the best of our knowledge, this study represents the first successful application of the AMBER 10 package to calculate QM/MM-PB/SA binding free energy. Our calculated binding free energy is in good agreement with the experimental results obtained for the c-Abl complex with Imatinib [41]. The results obtained from implementation of various QM potential methods show that DFTB method gives the most accurate binding free energy when compared to the experimental value. The reason for the disagreement with experiment might arise from the approximations involved in combining a QM/MM treatment for the protein ligand interaction with purely classical treatment of solvation, from approximations inherent to various methods and from the lack of a protein polarization term in these potentials. The accuracy of the QM/MM-PB/SA approach may be improved by optimizing the implicit solvent model and by using higher-level quantum mechanical methods. Our findings show a need for the consideration of electronic contributions to the binding affinity calculation which are frequently neglected during the classical MM-PB/SA calculations. The developed methodology provides a useful approach to atomic structure-based ligand-binding calculations and thereby opens up a new field of applications by permitting accurate computational studies of binding events where quantum mechanical effects play a major role.

The QM/MM-PB/SA approach presented here has a major advantage over purely classical-mechanics-based (MM-PB/SA) methods in that it does not require derivation of force field parameters for ligands. The free energy calculation procedure can thus be fully automated. This permits atomic-based binding affinity calculations for a wide range of ligands with satisfying accuracy and within reasonable CPU time.

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